

Effect of Nitrogen Sources on the AZO Dye Decolourization



Microbiology

KEYWORDS : Acid red 14, Bacillus species, yeast extract, ammonium chloride.

Suchita Dahiwade

C/O Dr. A. O. Ingle, P.G.T. D of Microbiology, L.I.T Campus, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur (M.S), India, Pin – 440033

Dr. A. O. Ingle

Associate Professor, P.G.T. D of Microbiology, L.I.T Campus, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur. Pin – 440033

Dr. S. R. Wate

Director, National Environmental Engineering Research Institute, Nehru Marg, Nagpur – 440020.

ABSTRACT

The isolated Bacillus species was found to decolourize commercial azo dye Acid red 14. The isolate could decolourize the azo dye upto 300 mg/l in minimal medium without nutritional supplements. Effect of various nitrogen sources beef extract, yeast extract, peptone at 0.05% and ammonium nitrate, sodium nitrate and ammonium chloride at 0.1 % and in combinations were studied on decolourization of the Acid red 14 by Bacillus species. 81% decolourization was observed in the presence of 0.1% of ammonium chloride in combination of 0.05% yeast extract as compared to other nitrogen sources. The decolourization was monitored for 72 h using the UV-Vis spectrophotometer.

Introduction

Contamination of the environment with hazardous and toxic chemicals is one of the major problems facing the industrialized nations today. Around 10^6 tonnes and more than 10,000 different synthetic dyes and pigments are produced annually world wide and used extensively in dye and printing industries. It is estimated that about 10% of these are lost in industrial effluents¹.

Synthetic dyes have a wide application in the food, pharmaceuticals, textile, leather, cosmetics and paper industries due to their ease of production, fastness and variety of colour compared to natural dyes. As synthetic dyes are relatively resistant to biodegradation, the elimination of coloured effluents in wastewater is based mainly on physical and chemical methods². The discharge of dyes into the environment impedes light penetration and then toxic to food chain organisms and to aquatic life.

Degradation of azo dyes by means of physical and chemical methods in waste water are recently done which are not viable options for treating large waste streams and cost prohibitive³. Living system, especially microorganisms can catalyze the degradation of wastes without disruption of the environment is represented⁴. The use of microorganisms for the removal of synthetic dyes from industrial effluents offers considerable advantages. Bacteria offer a cheaper and environment friendlier alternative for colour removal in textile effluents^{4,5}. The process is relatively inexpensive, it is a simple method and the running costs are low and the end products of complete mineralization are not toxic⁶. Their enzyme producing activity makes them effective decolourizers; they remove toxic metals by biosorption ultimately rendering the effluents more eco-friendly⁷.

Aerobic treatment of azo dye wastes though effective, is often the typical method of treatment⁸. Azo dyes generally resist aerobic microbial degradation, only organisms with specialized azo dye reducing enzymes were found to degrade azo dyes under fully aerobic conditions⁹. Aerobic metabolism of dyes by *Pseudomonas mendocina* M2M B-404 and *Sphingomonas xenophaga* BN6 is studied by Sarnaik and Kanekar (1999) and Stolz (1999), respectively^{10, 11}. Studies by Buitron et al. (2004) with Acid red 151 azo dyes under aerobic conditions using a microbial consortium led to 99% colour removal¹².

This research explores the ability of the bacteria isolated from the effluent samples to degrade the azo dye, Acid red 14 (AR14) in the minimal medium supplemented with different nitrogen sources.

Materials and Methods

Dyes and chemicals

The azo dye Acid Red 14 (AR 14) (C.I., 85% min dye content)

was kindly provided by The Dyestuff Manufacturing Association of India, Mumbai. The structure of the azo dye is as shown in the figure 1.

λ_{max} of the dye was determined by using UV-Visible Spectrophotometer (Aquamate/AQA 50303). The chemicals and medium components were purchased from Hi-Media labs, Bombay, India. All chemicals used were of analytical grade.

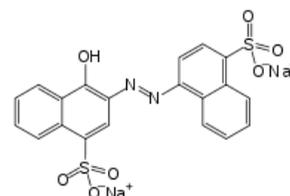


Figure 1: Azo dye - Acid red 14

Isolation , screening and selection of the bacteria :

Effluent samples collected from various industries from the local industrial area were subjected to the enrichment technique to isolate the AR14 decolourizing bacteria at 37 °C. Initially 32 cultures were isolated which were further screened for efficient dye decolourizers. The cultures were isolated on the nutrient agar using enrichment techniques. For the further studies the minimal medium (MM) used composed of (g/l) NaCl (1.0), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.1), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5), KH_2PO_4 (1.0), Na_2HPO_4 (1.0) at pH 7.0±0.2. Filter sterilized dye was used for decolourization studies. For the decolourization studies, precultured cells of the pure isolate were inoculated into the 100 ml flask containing 25 ml of the presterilized nutrient broth and cultured at 37°C with intermittent shaking. When the culture turbidity at 600nm was 1.0, the cells were diluted to 0.1 and the decolourization tests were done.

Decolourization assay :

Decolourization studies were carried out in minimal medium with Acid red 14 dye (dye concentration in the minimal medium was 20 mg/l) in the 150 ml Erlenmeyer flasks having 25 ml of the reaction mixture with intermittent shaking under aerobic conditions at 37±0.2 °C temperature and 7 pH. To study the effect of nitrogen sources, different organic nitrogen sources 0.05% (w/v) and inorganic nitrogen sources 0.1 % (w/v) were added to the minimal medium. Culture samples were withdrawn at defined intervals, and after centrifugation at 10,000 x g for 20 min at 10°C, the supernatants were used as samples for decolourization assay.

Analytical methods :

Decolourization of the dye colour in the minimal medium was determined by monitoring changes in the absorbance of the cul-

ture supernatant at wavelength 515 nm, and a control without inoculum was run under identical conditions. Whereas, the medium without dye and inoculum was used as blank. The percentage of decolourization was calculated from the ratio the absorption values of the inoculated effluent and the corresponding control without inoculum. All the experiments were performed in triplicates.

Results

When UV-visible spectra of the minimal medium with dye AR 14-containing culture after incubation for defined intervals were scanned from 200–800 nm, a significant decrease in optical density at 515 nm was observed, the decrease in the absorption values were noted up to 3 days. (Figure 2)

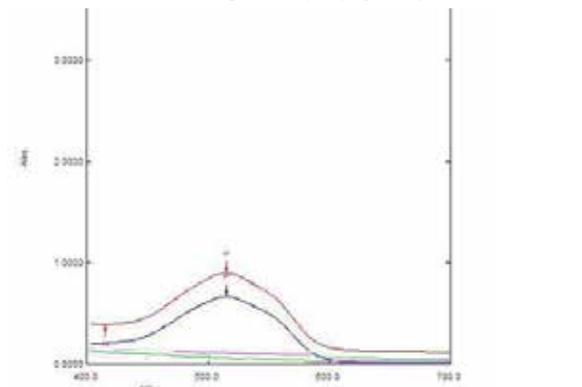


Figure 2 : Variations in the spectra showing decrease in concentration of Acid red 14 by isolated *Bacillus* species at different time intervals

The isolate could decolourize AR 14 dye in the minimal medium up to the dye concentration of 300 mg/l concentration. The cells of the pure culture did not show any adsorption of the dye on the microbial biomass. The isolated pure culture was *Bacillus* species (Accession no. HE962082).

Effect of various nitrogen sources on decolourization:

The effect of organic nitrogen sources and inorganic nitrogen sources on colour removal of dye effluent was investigated by adding different organic nitrogen sources namely beef extract, yeast extract and peptone at 0.05% and inorganic nitrogen sources namely ammonium nitrate, sodium nitrate and ammonium chloride at 0.1 % in combination of the best organic nitrogen sources amongst the three to the culture media. The percent decolourization was about 77% after 3 day’s incubation in the presence of 0.05% yeast extract, 64% in 0.05% of beef extract, 67.5% in 0.05% of peptone; whereas about 81% decolourization was observed in the presence of 0.1% of ammonium chloride in combination of 0.05% yeast extract as compared to other nitrogen sources (Figure 3).

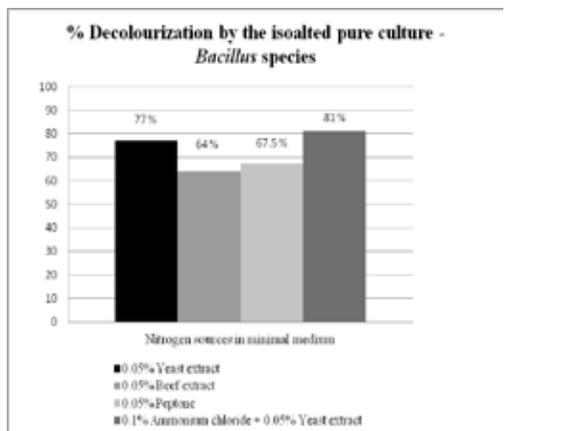


Figure 3: Comparative study of the percent decolourization by the isolated *Bacillus* species using different nitrogen sources

On the other hand there was no increase in cell density in the minimal medium containing ammonium nitrate and sodium nitrate eventually no cell growth , no decolourization.

Discussion

The efficiency of colour removal from dye effluent by this strain decreased with an increase in the concentration of dye effluent which was evident from the spectrophotometric data with maximum decolourization in the minimal medium having dye concentration up to 300 mg/l. This suggests that the concentration of a dye effluent affects the efficiency of colour removal. The significant decrease in the absorbance values of dye in this study was observed at 515 nm. This is in a good agreement with a previous report where yellow and red dyes in dye effluent peaked in the early part (400–450 nm) and the middle part (500–550 nm) of the spectrum measured between 400 and 700 nm, respectively, whereas blue and black dyes peaked in the end part (600–650 nm) of the spectrum¹³.

Although, it is a known fact that microorganisms are present in the contaminated sites or in effluents but their action need bi-stimulation which usually involves the addition of nutrients and oxygen to help indigenous microorganisms. These nutrients are the basic building blocks of life and allow microbes to create the necessary enzymes to break down the contaminants.

Azo dye decolourization by mixed as well as pure cultures generally requires complex organic sources, such as yeast extract, peptone, beef extract or a combination of complex organic sources and carbohydrates^{14, 15}. Regeneration of NADH plays a very important role, it acts as electron donor for the reduction of azo dyes in the effective decolourization of azo dyes by microorganisms. Yeast extract previously has been shown to supplement the growth of azo dye degrading bacteria¹⁶. The effects of nutrient supplementation of either glucose or peptone enhanced decolourizing activity^{17, 18, 19, 20}.

It has also been seen that the *Bacillus* sp. ADR degrade and tolerates the higher concentration of azo dyes using only yeast extract as sole carbon and nitrogen source. Similarly, *Enterobacter* sp. azol could not metabolize the dye as sole carbon and energy source but it needed either glucose or peptone as co-substrate for decolourization¹⁷. Yeast extract (representing the growth factor) required to activate the necessary coenzyme-producing metabolic pathways for azoreductase activities are the key components for the decolourization activity²¹.

Another interesting observation in this study was that the combination of yeast extract and ammonium chloride enhanced the decolourization of AR 14 dye. The electron donors or co-substrates are generally added to the minimal medium to enhance the decolourization efficiency of the microorganisms in the system.

Mabrouk et al. (2008) isolated a dye-decolourizing bacterium and identified as *Bacillus subtilis* HM by 16S rDNA sequencing. The bacterium was able to decolourize aerobically eight different sulfonated azo dyes. Omitting both carbon and nitrogen sources from the medium caused 57.6% decrease in Fast red colour removal. Under a near – optimal conditions, almost 99 % decolourization was obtained after 6 h²².

Although decolourization is a challenging process to the wastewater treatment, the result of this findings and literature suggest a great potential for bacteria to be used to remove colour from dye waste. Thus, biological processes that are simple, fast and economical can be adopted for treating the coloured wastewater.

However, further studies are needed to identify the exact biochemical processes involved in the decolourization of dyes. May be the combination of microorganisms, chemical and physical techniques to enhance the efficiency of the microbiological decomposition can be expected in future²³.

Acknowledgement:

The authors would like to extend their gratitude to PGTD of Microbiology, R.T.M Nagpur University, Nagpur and NEERI, Nagpur for funding and extending their helping hands to carry out the experimental work.

REFERENCE

- 1) Young L, Yu J (1997). Ligninase catalysed decolorization of synthetic dyes. *Water Res.* 31: 1187-1193. | 2) Banat IM, Nigam P, Mc Mullan G, Marchant R, Singh D (1996). Microbial decolorization of textile dye containing effluents. A Review. *Biore Technol* 58: 217-27. | 3) Do T, Shen J, Cawood G, Jeckins R (2002). Biotreatment of textile effluent using *Pseudomonas* spp. Immobilized on polymer supports", In: *Advances in biotreatment for textile processing*. Hardin, I.R; Akin D.E and Wilson J.S (Eds). University of Georgia Press.; Maier et al., 2004 | 4) Muurugalatha N, Mohankumar A, Sankaravadiwo A, Rajesh C (2010). Textile effluent treatment by *Bacillus* species isolated from processed food. *African Journal of Microbiology Research*. 4(20): 2122-2126 | 5) Olukanni OD, Osuntoki AA, Gbenle GO (2006). Textile effluent biodegradation potentials of textile effluent-adapted and non-adapted bacteria. *Afr. J. Biotechnol.*, 5(20): 1980-1984. | 6) Forgas E, Cserhati T, Oros G (2004). Removal of synthetic dyes from waste waters. *Environ. Inter.*, 30: 953-971. | 7) Erkurt EA, Unyayar A, Kumbur H (2007). Decolorization of synthetic dyes by white rot fungi, involving laccase enzyme in the process. *Process Biochem.*, 42: 1429-1435. | 8) Seshadri S, Bishop PL, Agha AM (1994). Anaerobic / Aerobic treatment of selected azo dyes in waste water. *Water Manage.*, 14: 127-137. | 9) Ganesh R, Boardman GD, Michelsen D (1994). Fate of azo dyes in sludges. *Water Res.* 28(6): 1367-1376. | 10) Sarnaik S, Kanekar P (1999). Biodegradation of methyl violet by *Pseudomonas mendocina* MCM B -402. *Appl. Microbiol. Biotechnol.* 52: 251-254. | 11) Stolz A (1999). Degradation of substituted naphthalene sulfonic acids by *Sphingomonas xenophaga* BN6. *J. Ind. Microbiol. Biotechnol.* 23 (4-5): 391-399. | 12) Buitron G, Quezada M, Moreno G (2004). Aerobic degradation of the azo dye acid red 151 in a sequencing batch biofilter. *Bioreour. Technol.* 92: 143-149. | 13) O'Neill C, Hawkes F.R, Hawkes D. L., Lourenço N. D., Pinheiro H. M. and Deléé, W., 1999. "Colour in textile effluents - sources, measurement, discharge consents and simulation: a review", *J. Chem. Technol. Biotechnol.*, 74 : 10009-1018. | 14) Chen K. C., Wu J.Y, Liou D. J., and Hwang S. C. J., 2003. "Decolorization of the textile dyes by newly isolated bacterial strains", *J. Biotechnol.*, 101 : 57. | 15) Khehra M. S., Saini H. S., Sharma D. K., Chadha B. S., and Chimni S. S., 2005. "Comparative Studies on Potential of Consortium and Constituent Pure Bacterial Isolates to Decolorize Azo Dyes", *Water Res.*, 39 : 5135. | 16) Sponza D. T., and Isik M., 2002. "Decolorization and azo dye degradation by anaerobic/aerobic sequential process", *Enzyme and Microbial Technology*, 31: 102-110. | 17) Jirasripongpun K., Nasanit R., Niruntasook J., and Chotikasatjan B., 2007. "Decolorization and Degradation of C. I. Reactive Red 195 by *Enterobacter* sp.", *Thammasat Int. J. Sc. Tech.*, 12 (4). | 18) Carliell C.M., Barclay S.J., Shaw C., Wheatley A.D., Buckley Q.A., 1998. "The Effect of Salts used in Textile Dyeing on Microbial Decolourisation of a Reactive Azo dye", *Environ. Technol.*, 19 : 1133-1137. | 19) Chen K.C., Wu J.Y., Liou D.J., Hwang S.J., 2003. "Decolorization of the textile dyes by newly isolated bacterial strains", *J. Biotechnol.*, 101 : 57-68. | 20) Ambrosio S.T., Campos-Takaki G. M., 2004. "Decolorization of reactive azo dyes by *Cunninghamella elegans* ucp 542 under co-metabolic conditions", *Bioreour. Technol.*, 91 : 69-75. | 21) Chang J., Chou C., Lin Y., Lin P., Ho J., and Hu T., 2001b. "Kinetic characteristics of bacterial azo-dye decolorization by *Pseudomonas luteola*", *Wat. Res.*, 35(12) : 2841-2850. | 22) Mabrouk M. E. M., and Hoda H., Yusef., 2008. "Decolorization of Fast Red by *Bacillus subtilis* HM", *Journal of Applied Sciences Research*, 4(3): 262-269. | 23) Forgasca E., Cserhati T., Oros G., 2004. "Removal of synthetic dyes from wastewaters: a review", *Environment International*, 30 : 953- 971. |